Trichloroethylene

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

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Mijnheer de staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om naast het afleiden van gezondheidskundige advieswaarden ook te adviseren over andere onderwerpen ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In 1995 heeft de Staatssecretaris van Sociale Zaken en Werkgelegenheid besloten tot het opstellen van een zogenaamde niet-limitatieve lijst van voor de voortplanting vergiftige stoffen. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1, 2 en 3 wat betreft effecten op de voortplanting en stoffen die schadelijk kunnen zijn voor het nageslacht via de borstvoeding. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In dit kader bied ik u hierbij een advies aan over trichloorethyleen. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb deze publicatie heden ter kennisname aan de Minister van Volksgezondheid, Welzijn en Sport en aan de Minister van de Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

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Trichloroethylene

Evaluation of the effects on reproduction, recommendation for classification

Committee for Compounds toxic to reproduction,
a committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2003/09OSH, The Hague, 22 December 2003

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The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature and Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondsheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie trichloorethyleen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn, en dat voldoende diergegevens laten zien dat trichloorethyleen de fertiliteit niet schaadt. Zij adviseert daarom om trichloorethyleen niet te classificeren.
- Voor effecten op de ontwikkeling adviseert de commissie trichloorethyleen in categorie 2 (*stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken*) te classificeren en met T;R61 te kenmerken.
- Voor effecten tijdens lactatie, adviseert de commissie om trichloorethyleen niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends to classify compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed trichloroethylene.

The committee's recommendations are

- For effects on fertility, the committee recommends not classifying trichloroethylene on the basis of a lack of sufficient human data, and sufficient animal data which show that no classification is indicated.
- For developmental toxicity, the committee recommends classifying trichloroethylene in category 2 (*substances which should be regarded as if they impair fertility in humans*) and labelling trichloroethylene with T;R61.
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data trichloroethylene should not be labelled.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds Toxic to Reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1, 2 or 3) or labelled as may cause harm to breastfed babies (R64).

1.2 Committee and procedure

The present document contains the classification of enflurane by the Health Council's Committee for Compounds Toxic to Reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by dr ir APM Wolterbeek and ir DH Waalkens-Berendsen, of the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concern-

ing adverse effects with respect to fertility and development and lactation of the above mentioned compound.

Classification and labelling was performed according to the guidelines of the European Union listed in Annex C.

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In 2002, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, the committee has agreed upon a number of additional considerations.

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.

- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur at a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols* for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceedence of the exposure limit for the general population, eg the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 2000. References publiced between 2000 and November 2003 were no reason for the committee to adjust her recommendations. Literature was selected prima-

* Organisation for Economic Cooperation and Development

rily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited.

The committee chose to describe human studies in the text, starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

Animal data are described in the text and summarised in Annex D.

1.6 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- Lack of appropriate data preclude assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxic to reproduction is indicated.

1.7 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter 2

Trichloroethylene

2.1 Introduction

Name	:	Trichloroethylene
CAS-no	:	79-01-6
Synonyms	:	ethinyl trichloride, TCE, trichloroethene, ethylene trichloride, 1,1,2-trichloroethylene, 1,1,2-trichloroethene, acetylene trichloride, trilene
Use	:	organic solvent, dry cleaning, degreasing metals, previously used as anaesthetic
Mol weight	:	131.4
Chem formula	:	C_2HCl_3
Conversion factor	:	1 ppm = 5.37 mg/m^3 (101 kPa, 25ºC)

Inhaled doses of trichloroethylene are metabolised extensively in humans. Percentages of the dose metabolised have been reported to be between 40% and 75% of the retained dose. Trichloroacetic acid (TCA) is one of the principle oxidative metabolites of trichloroethylene in humans. The other principal metabolites in humans are trichloroethanol and trichloroethanol-glucuronide (ATS97).

2.2 Human studies

Fertility

Rasmussen *et al*. (Ras88) evaluated the semen of a group of metal workers (n=13) occupationally exposed to high doses (degreasing metals with trichloroethylene for more than 20 hours per week) of trichloroethylene for more than 20 hours per week. Fourteen non-exposed physicians, working at university institutions, served as controls. There was no difference between the two groups in terms of sperm count or morphology, but in the exposed group a slightly higher (statistically insignificant) prevalence of mature spermatozoa containing two fluorescent Y bodies was observed, which may indicate Y-chromosomal nondisjunction.

Taskinen *et al*. (Tas89) conducted a nested case-control study of 120 cases of spontaneous abortion and 251 controls on the basis of a file of 6000 Finnish workers who had been biologically monitored for exposure to six organic solvents (styrene, xylene, toluene, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane) at the Finnish Institute of Occupational Health during 1965-1983. Information about their marriages, their wives' pregnancies and spontaneous abortions were obtained from national registries; data on paternal occupational exposure to solvents were collected by means of a questionnaire sent to workers and covered the period of spermatogenesis. No association was found between paternal occupational exposures to trichloroethylene and the incidence of spontaneous abortion (cases n=17, referents=35; crude odds ratio 1.0; (CI 0.6-2.0)). Furthermore, no effect of paternal exposure to halogenated hydrocarbons was observed on the incidence of spontaneous abortions.

Sallmén *et al*. (Sal95) performed a retrospective time to pregnancy study among women biologically monitored for exposure to six organic solvents (styrene, xylene, toluene, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane) at the Finnish Institute of Occupational Health during 1965-1983 (n=3265). In this study 197 women participated. More than half of the subjects (105) were exposed to organic solvents during their time to pregnancy. Nearly a quarter were highly exposed (handling solvents daily or 1-4 days a week supported by individual exposure measurements). Exposure to organic solvents was significantly related with reduced fecundity after adjustment for confounding factors (incidence density ratio of clinical pregnancies was 0.69 (95% CI 0.48-0.99) and 0.41 (95% CI 0.27-0.62) for low and high exposure, respectively). The incidence density ratios for workers exposed to trichloroethylene were 1.21 (CI 0.73-2.00, low exposure, n=19) and 0.61 (CI 0.28-1.33, high exposure, n=9). However, a statistically significant relation was shown between high exposure to halogenated hydrocarbons and reduced fecundity (incidence density ratio 0.53 (95% CI 0.29-0.97, n=15).

Chia *et al*. (Chi96) examined the association between exposure to trichloroethylene and effects on spermatogenesis among a group of workers in an electronics factory. A total of 85 workers were included in the study. Semen analysis included volume, density, viability, motility and morphology. Personal monitoring of environmental trichloroethylene exposure was conducted for 12 workers and urine, collected on the end of the day in which the semen given, was analysed for trichloroacetic acid. The mean personal exposure levels of trichloroethylene among the 12 workers was 160 mg/m^3 (range 63 - 704 mg/m^3). The mean trichloroacetic acid levels in the urine was 22.4 mg/g creatinine (range 0.8 - 136.4). Since there was no non-exposed control group in this study, the results were compared with WHO criteria. Compared to these criteria, 71.8% of the subjects had a normal semen volume, sperm density of 88.2% of the subjects was normal and the sperm motility of 64.7% of the subjects was normal. Sperm morphology was normal in 30.6% of the subjects. There were no differences in volume, motility and morphology among the high-exposure (urine trichloroacetic acid concentration > 25 mg/g creatinine) and low-exposure (urine trichloroacetic acid concentration < 25 mg/g creatinine) groups. The prevalence rate ratios of hyperzoospermia were higher with increasing urine trichloroacetic acid levels suggesting a dose-response relationship. In the same group of workers serum concentrations of several hormones were measured (Chi97, Goh98). Except for a positive correlation between urine levels of trichloroacetic acid and insulin levels (Goh98) there were no consistent relationships between the urine concentration of trichloroacetic acid and serum levels of hormones.

Development

In a case-referent study of Kurppa *et al*. (Kur83) the relationship between exposure to organic solvents and the incidence of congenital malformations was investigated. Data were derived from the Finnish Register of Congenital Malformations. Initial two-year data showed an association between maternal exposure to organic solvents and defects of the central nervous system among children born to these mothers (14 case and 3 referent mothers had been exposed to solvents in early pregnancy). However, for the following three-year period this association was no longer detectable (respective distribution: 6 cases and 6 referents).

Goldberg *et al*. (Gol90) studied the association between congenital heart malformations and drinking water contaminations. This study was based on a previous observation showing that one third of the children with congenital heart diseases in Tucson, Arizona came from a small area in the southwestern part of the city in which drinking water was found to be contaminated with trichloroethylene (>5 µg/l) and to a lesser extent with dichloroethylene and chromium. From interviews with 707 parents having children with congenital heart defects (246 consumed the contaminated water and 461

were not exposed) an increased prevalence of congenital heart diseases among children of mothers who consumed the contaminated water (0.68%) compared to the children of mothers who lived outside the area (0.26%; $p<0.001$) was shown. The ratio decreased to near unity for new arrivals in the contaminated area after closure of the well.

Lindbohm *et al*. (Lin90) studied the association between medically diagnosed spontaneous abortions and maternal occupational exposure to organic solvents. The final population for the analysis was restricted to the matched case-control sets, who confirmed their pregnancy and reported in detail their occupational exposures during early pregnancy (73 cases of spontaneous abortion and 167 controls). The incidence of spontaneous abortions was increased among the women exposed to organic solvents (58%) compared to controls (42%); odds ratio 2.2 (95% CI 1.2-4.1). The association between trichloroethylene exposure and the incidence of spontaneous abortions was negative; the odds ratio, adjusted for confounding factors, was 0.6 (95% CI 0.2-2.3).

Windham *et al*. (Win91) performed a case control study in California using women who had a spontaneous abortion at less than 20 weeks gestation each matched to two controls who had had a live birth. The incidence of spontaneous abortions among women exposed to trichloroethylene was increased (crude odds ratio 3.1 (95% CI 0.92-10.4). However, these results were based on only 6 cases and 4 controls, and 4 of these women were also exposed to tetrachloroethylene.

Information on 7316 pregnancies was obtained from the hospital discharge register for 9186 women identified as working in Finnish laboratories (Tas94). The pregnancies resulted in 5663 births, 687 spontaneous abortions and 966 induced abortions. A case-referent study was conducted within this cohort. The 206 women with only one registered spontaneous abortion and 329 controls randomly selected among women who had given birth to a normal infant were included in the analysis of spontaneous abortion. Seven women having a spontaneous abortion and nine controls reported exposure to trichloroethylene (the odds ratio, adjusted for confounding factors, was 1.6 (95% CI 0.5-4.8).

A cross-sectional study was conducted by Bove *et al*. (Bov95) using environmental and birth outcome databases. The study focused on 75 towns in an area in nothern New Jersey where some water supplies were contaminated. A total of 80938 live births and 594 fetal deaths were studied. The control group (n=52334) comprised of all live births from the study population showing no adverse pregnancy outcome. An positive (not statistically significant) association was found between trichloroethylene levels of >10 ppb and oral clefts (n=3 cases, odds ratio 1.30 (90% CI 0.39-3.68)), central nervous system defects (n=6 cases, odds ratio 1.68 (90% CI 0.76-3.52)) and neural tube defects (n=4 cases, odds ratio 2.53 (90% CI 0.91-6.37)).

Lactation

Fisher *et al*. (Fis97) studied the human blood/air and milk/air partition coefficient (PC) in human blood and human milk samples. The objective of this study was to evaluate the potential chemical exposure of a nursing infant by ingestion of contaminated milk from a mother who was occupationally exposed to vapours; To estimate infant's exposure, a generic human pharmacokinetic (PB-PK) lactation model was developed. The model was based on an 8-hour exposure period of the mother to a constant vapour concentration equal to the threshold limit value for trichloroethylene of 50 ppm (= 268.5 mg/m^3). The experimentally determined blood/air and milk/air PC values were used in the PB-PK lactation model. The predicted amount of trichloroethylene ingested by a nursing infant over a 24-hour period was 0.496 mg in 0.92 l (0.54 mg/l).

2.3 Animal studies

Table 1 and 2 (Annex D summarizes fertility and developmental studies with trichloroethylene in animals).

Fertility studies

Inhalation

Beliles *et al*. (Bel80) exposed rats and mice to 0, 100 or 500 ppm trichloroethylene (0, 537 and 2685 mg/m^3) for 5 consecutive days 7 hours per day. One, 4 and 10 weeks after dosing, animals were sacrificed for the collection of caudal epididymal sperm. In rats, no effect of trichloroethylene on sperm morphology was observed. In mice, the incidence of abnormal sperm was dose dependently increased at week 1 and 4. No effect was observed at week 10.

Groups of 50 male NMRI mice were exposed to 0, 50, 202 and 450 ppm trichloroethylene (0, 269, 1085, 2417 mg/m^3) for 24 hours (Sla80) by inhalation. General toxic effects were not observed. After the 24 hour exposure period, each treated male was mated with 1 untreated female for 4 consecutive days. Females were changed every 4th day, alltogether 12 times and sacrificed 13 days after removal from the males. No effects were observed on fertility rates, pre-or post-implantation loss and dominant lethal mutations.

Land *et al*. (Lan81) exposed (C57B1/C3H)F_1 male mice 4 hours/day during 5 days to trichloroethylene (0, 200 and 2000 ppm = 0, 1074, 10740 mg/m^3) by inhalation. Data about general toxic effects were not presented. Twenty eight days after the first day of exposure epididymal sperm was examined. A statistically significant increase in the

incidence of abnormal spermatozoa was found in the highest dose group (2.43% versus 1.42% in the control group).

Gavage

Manson *et al*. (Man84) exposed female Long Evans rats by gavage during 2 weeks pre-mating, 1 week mating (5 days a week) and 3 weeks gestation (7 days a week) to trichloroethylene in corn oil at exposure levels of 0, 10, 100 and 1000 mg/kg body weight/day. Treated females were mated with untreated males. Animals of the high dose group showed maternal toxic effects: 3 animals died and weight gain was significantly reduced. No effect of trichloroethylene exposure was observed on the length of the oestrus cycle nor on fertility.

Zenick *et al*. (Zen84) exposed male Long Evans rats to 0, 10, 100 and 1000 mg trichloroethylene/kg body weight by gavage for 6 weeks (5 days a week). At the end of week 1 and 5 of the exposure period and 4 weeks post-exposure, males were allowed to mate with ovariectomized hormonally primed females and copulatory behaviour and semen evaluations were conducted. In week 1, reduced body weight gain, increased relative liver weight, and impaired copulatory behaviour were observed among the animals of the highest dose group. In week 5, copulatory performance returned back to normal. Semen evaluations did not show any spermatotoxic effect. Furthermore, no effect was observed on testosterone levels. These results were confirmed in a study of Nelson and Zenick (Nel86) in which increased ejaculation latency was seen following oral administration of trichloroethylene at a level of 1000 mg/kg body weight. Since naltrexone, an opiate antagonist, blocked this effect the authors stated that the alterations in copulatory behaviour may be attributed to the narcotic properties of trichloroethylene.

Diet

Lamb *et al*. reported the results of dietary exposure to trichloroethylene on reproduction, fertility and development in Swiss CD-1 mice (Lam97a) and Fischer F344 rats (Lam97b) using the Reproductive Assessment by Continuous Breeding (RACB) protocol. Trichloroethylene microencapsulated in a gelatin and sorbitol shell was added to the diet at concentrations of 0, 1.5, 3.0 and 6.0 g/kg diet resulting in a trichloroethylene intake of 0, 100, 300 and 700 mg/kg body weight and 0, 76, 156 and 289 mg/kg body weight for mice and rats, respectively.

In male and female mice of the highest dose group of both generations hepatic and/or renal toxicity was observed (increased organ weights and histopathological lesions). Furthermore, in the F1 generation mortality was increased in the highest dose group. There were no treatment related effects on mating, fertility and reproductive perfor-

mance in either the F0 or F1 generation, but sperm motility was reduced by 45% in F0 males and 18% in F1 males of the highest dose group.

In rats body weights were decreased in F0 females of all dose groups and in F0 males of the highest dose group. Kidney and liver weights were increased in F0 males and females of the highest dose group. In the F1 generation body weights of male and females were decreased in all dose groups whereas the liver weight was increased. Testis weight was decreased in all dose groups affected but no histopathological changes were observed.

Development

Inhalation

Schwetz *et al*. (Sch75) studied the developmental effects of trichloroethylene (300 ppm = 1611 mg/m^3) by inhalation during gestational days 6-15, 7 hours/day in Sprague Dawley rats and Swiss Webster mice. In rats maternal body weights were slightly, but statistically significantly, decreased. In mice no effects on maternal body weights were observed. In both species, no significant maternal, embryonal, foetal toxic or teratogenic effects were observed.

Dorfmueller *et al*. (Dor79) exposed 4 groups of female Long-Evans rats to filtered air or to 1800 ppm (9666 mg/m^3) trichloroethylene, 6 hours/day using different treatment regimes. During a 2 week premating period 2 groups of rats were treated with air and the other 2 groups were treated with trichloroethylene for 5 days per week. During the first 20 days of gestation one group of each two groups was treated to air and the other to trichloroethylene for 7 days per week. In the group exposed during pregnancy alone, the incidences of skeletal (incomplete ossification sternum) and soft-tissue (displaced ovaries) anomalies were statistically significantly increased, indicative of delayed foetal development. Furthermore, the committee concludes that except for a reduction in post-natal body weight of the offspring of mothers pregestationally exposed, no treatment-related maternal toxicity, embryotoxicity, severe teratogenicity or significant behavioural deficits were observed in any of the treatment groups.

In a study of Beliles *et al* (Bel80, see also section about fertility) female Sprague Dawley rats were exposed to trichloroethylene (0, 500 ppm = 0, 2685 mg/m^3) during a pregestational period of 3 weeks (during these weeks half of the groups were exposed) and during gestation day 0-18 or 6-18 for 5 days a week, 7 hours a day. No maternally toxic or developmental effects were observed. In the same study, New Zealand White rabbits were exposed to 500 ppm trichloroethylene during gestation days 0-21 or 7-21 with or without a 3 weeks pregestational exposure period (5 days per week, 7 hours a day). No maternally toxic, foetal toxic or teratogenic effects were observed, except for 4

foetuses in 2 litters of one group which showed external hydrocephaly. The authors stated that the occurrence of this most unusual anomaly, while not statistically significant, could not be discounted entirely as occurring by chance.

Healy *et al*. (Hea82) exposed Wistar rats to trichloroethylene (0 and 100 ppm = 0 and 537 mg/m^3) for 4 hours/day from day 8-21 of gestation. The incidence of dams with total resorptions was 21.9% in the exposed group and 6.5% in the control group, respectively. No external, visceral or skeletal malformations were found, but a delay in foetal skeletal maturation was suggested by reduced foetal weight and by an increase in bipartite or absent ossification centres in the trichloroethylene-exposed group.

Carney *et al* (Car01) exposed CD rats by inhalation to 0, 50, 150 and 600 ppm trichloroethylene (0, 269, 806 and 3222 mg/m^3) for 6 hours per day, 7 days per week from gestation day 6-20. Dams exposed to 600 ppm showed a decrease in weight gain (22% less that the control group) on gestation day 6-9. No visceral and skeletal malformation were observed.

Gavage

Organic materials concentrated from the drinking waters of five US cities selected as representative of the major sources of raw water were administered to groups of pregnant CD-1 mice from GD 7-14 by gavage at dose levels representative for 300, 1000 and 3000 times the anticipated human exposure to these materials (trichloroethylene exposure level in the highest dose group was calculated to be 0.02 mg/kg body weight /day. In the drinking-water concentrates also other compounds, e.g. chloroform [calculated exposure level 7.1 mg/kg body weight], were present). The dams were killed on GD 18 and the foetuses were examined for skeletal and visceral anomalies (Kav79). Except from slight effects on body weights and relative liver weight, no maternal toxic effects were observed. No effects were observed on the foetuses.

Manson *et al*. (Man84) exposed female Long Evans rats by gavage during 2 weeks premating, 1 week mating (5 days per week) and 3 weeks gestation (7 days per week) to trichloroethylene in corn oil at exposure levels of 0, 10, 100 and 1000 mg/kg body weight/day. Treated females were mated with untreated males. Animals of the high dose group showed maternal toxic effects; 3 animals died and weight gain was significantly reduced. In this group pup mortality was increased (7.7%, 7.0%, 6.4% and 16.9% in the 0, 10, 100 and 1000 mg/kg body weight group, respectively) with the majority of deaths occurring among female pups at birth.

Smith *et al*. (Smi89) exposed Long Evans rats to 0, 330, 800, 1200 or 1800 mg/kg bw trichloroacetic acid (one of the major metabolites of trichloroethylene) by gavage on days 6-15 of gestation. Maternal weight gain was reduced at 800, 1200 and 1800 mg/kg and a dose-related increase in spleen and kidney weights was observed which was statis-

tically significant at all dose levels. Embryolethality was significantly increased in the three highest dose groups. Foetal weight and foetal length were statistically significantly dose-related decreased in all treatment groups. The incidence of soft tissue malformations, mainly in the cardiovascular system (interventricular septal defect and levocardia), was dose-related and statistically significantly increased in all dose groups. The incidence of skeletal malformations was statistically significantly increased in the 1200 and 1800 mg/kg groups.

In a study of Cosby *et al*. (Cos92), female B6D2F1 mice were treated by gavage from GD 1-5, 6-10 or 11-15 with trichloroethylene at 0, 1% and 10% of the oral LD_{50} (= 2402 mg/kg). No maternal, reproductive or developmental effects were observed at either dose level and either exposure period.

Fisher *et al* (Fis01) treated pregnant CDR(CD) Sprague Dawley rats by oral intubation with 500 mg/kg bw trichloroethylene, 300 mg/kg bw trichoroacetic acid or dichloroacetic acid (300 mg/kg bw) once per day on days 6 through 15 of gestation. Fetal hearts were examined on (GD)day 21. Both trichloroacetic acid and dichloroacetic acid caused a modest decrease in maternal weight gain (3% and 8% respectively). No effects on maternal weight was observed after exposure to trichloroethylene. Fetal weights were decreased after treatment with trichloroacetic acid and dichloroacetic acid, 8% and 9% respectively. The heart malformation incidence for fetuses from dams treated with trichloroethylene, trichloroacetic acid and dichloroacetic acid did not differ from the control values on a per fetus or per litter basis. No differences were found between the control and treated groups on early resorptions, number of implantations and mean litter size.

Diet

Lamb. *et al*. reported the results of dietary exposure to trichloroethylene on reproduction, fertility and development in Swiss CD-1 mice (Lam97a) and Fisher F344 rats (Lam97b). Details of this study are already described in the section about fertility.

In mice, a 4% reduction in pup body weights was observed and maternal trichloroethylene exposure during lactation was associated with a significant increase in perinatal mortality (mortality incidence was 28% and 61% in the control and high dose group, respectively). After weaning no effect on mortality was observed.

In rats, in the F0 generation the number of pups per litter was decreased by 9 and 16% in the mid and high dose group, respectively. In the F1 generation, pup weights were decreased in all dose groups.

Drinking water

Taylor *et al*. (Tay85) exposed female Sprague Dawley rats to 0, 312, 625 or 1250 mg/l trichloroethylene in the drinking water for 14 days prior to mating, during gestation and up to lactation day 21. Data about general toxicity were not presented. Behavioural tests of the offspring showed that locomotor activity was significantly higher in 60-day old male offspring of the high dose group and exploratory behaviour was higher in 60- and 90-day old male offspring of all trichloroethylene groups compared to control.

Dawson *et al*. (Daw93) studied foetal cardiac defects in Sprague Dawley rats induced after exposure to trichloroethylene (0, 1.5 and 1100 mg/l in drinking water 2 months before gestation only, 2 months before gestation and during gestation, or during gestation alone. When trichloroethylene was applied exclusively in the period before gestation no effects were observed. Trichloroethylene given before and during gestation induced cardiac effects at both dose levels. However, this effect was not dose dependent. When administered exclusively during the gestation period, only the high dose produced a significant increase in cardiac effects. No maternal effects or other congenital malformations were observed.

Johnson *et al*. (Jon98) studied the effect of various trichloroethylene metabolites on foetal cardiac teratogenic effects in rats. Sprague Dawley rats were given drinking water containing these metabolites during pregnancy. No signs of general toxicity were observed in any of the treated groups. Furthermore, no differences were observed in reproductive and noncardiac developmental parameters between control and treatment groups except for an increased number of implantation sites and resorptions sites in the trichloroacetic acid treated group (2730 mg/l). Moreover, foetuses receiving this compound were the only ones demonstrating a significant increase in cardiac defects.

Lactation

Fisher *et al*. (Fis90) performed an inhalation and a drinking water study with rats to gain data for the development of a PB-PK model to describe the kinetics of trichloroethylene in the lactating rat. In the inhalation study, lactating rats were exposed to 600 ppm (3222 mg/m^3) trichloroethylene for 2 weeks (4 hours a day, 5 days a week). On day 11, 20 hours after exposure, milk was collected for analysis of trichloroacetic acid and on day 14 milk was collected immediately after exposure for analysis of trichloroethylene and trichloroacetic acid. The measured concentration of trichloroethylene was about 110 mg/l milk (the highest predicted concentration was about 220 mg/l). The measured concentration of trichloroacetic acid in milk was about 2.5 mg/l on day 11 and about 6 mg/l on day 14 (the highest predicted concentration was about 9 mg/l). In the drinking water study lactating rats were given water containing 333 mg/l trichloroethylene for 3 weeks

(5 days a week). On day 13, 14 and 21 milk was collected for trichloroethylene and trichloroacetic acid analysis. The concentration of trichloroethylene was below the detection limit, the concentration of trichloroacetic acid in milk was about 0.4, 0.6 and 1.5 mg/l on day 13, 14 and 21, respectively.

2.4 Conclusion

The human studies on the potential effects of occupational exposure to trichloroethylene on fertility did not show significant effects (Ras88, Tas89, Sal95, Chi96) or the results of the studies were inconsistent and difficult to interpret (Chi97, Goh98).

In a study of Beliles *et al*. (Bel80), the incidence of abnormal sperm cells was dose-dependently increased in mice 1 and 4 weeks after inhalatory exposure to trichloroethylene. After 10 weeks, no effects were observed. In rats, trichloroethylene had no effect on sperm morphology. In an inhalatory study with rats of Slacik-Erben *et al*. (Sla80), no effects on fertility were observed. Land *et al*. (Lan81) showed an effect of inhalatory trichloroethylene exposure (10740 mg/m^3) on the incidence of abnormal spermatozoa in mice (2.43% versus 1.42% in the control group) but the incidence of abnormal sperm in the exposed group is still very low. Manson *et al*. (Man84) showed no effect on female fertility in Long Evans rats treated by gavage with trichloroethylene at a dose level inducing general toxicity. Zenick *et al*. (Zen84) and Nelson and Zenick (Nel86) observed an impaired copulatory behaviour of male Long Evans rats treated with trichloroethylene at general toxic and narcotic dose levels. In the studies described by Lamb *et al*. (Lam97a, Lam97b) effects of dietary trichloroethylene were observed on reproductive parameters at dose levels inducing general toxic effects.

In conclusion, based on the human studies the committee recommends not to classify trichloroethylene with respects to effects on fertility because of a lack of appropriate data. Based on the data from the animal studies, the committee is of the opinion that sufficient data show that no classification for effects of trichloroethylene on fertility is indicated.

Epidemiological studies of Kurppa *et al*. (Kur83), Lindbohm *et al*. (Lin90) and Taskinen *et al*. (Tas94) did not show significant effects of trichloroethylene exposure on human development. Goldberg *et al*. (Gol90) showed an effect of trichloroethylene contaminated drinking water consumption during the month before conception and the first trimester of pregnancy and the incidence of congenital heart diseases. However, exposure data were not clearly defined and exposure to other substances in the drinking water had occurred. Bove *et al*. (Bov95) found an association between consumption of drinking water contaminated with trichloroethylene and the incidence of oral clefts, central ner-

vous system defects and neural tube defects. Limitations to this study were the uncertainty regarding exposure classification and the small number of cases.

Windham *et al*. (Win91) showed a significantly increased incidence of spontaneous abortions among women exposed to trichloroethylene. However, a consistent dose-relationship was not observed, the number of cases and controls was very small and most of the women were exposed to a variety of solvents.

Schwetz *et al*. (Sch75) did not show developmental effects in rats and mice exposed to trichloroethylene by inhalation at a concentration of 1611 mg/m^3. No developmental effects were observed in rats after exposure to 0, 269, 806 and 3222 mg/m^3 qs well (Car01). Dorfmueller *et al*. (Dor79) showed a delayed development, but no embryotoxic, teratogenic or behavioural effects were observed, in rats exposed to trichloroethylene by inhalation at a concentration of 9666 mg/m^3. In a developmental study of Beliles *et al*. (Bel80) no effect of inhalatory exposure of trichloroethylene in rats was observed but in rabbits some foetuses showed (external) hydrocephaly. An increased incidence of dams with total resorptions and pups showing a delayed skeletal development was observed by Healy *et al*. (Hea82) in rats treated with trichloroethylene at a (relative low) concentration of 537 mg/m^3 by inhalation. However, in the control group resorptions were observed as well. Manson *et al*. (Man84) showed an effect on pup mortality in Long Evans rats treated by gavage with trichloroethylene at a dose level inducing maternal toxicity. Smith *et al*. (Smi89) observed embryolethality, reduced foetal weight and foetal length and visceral and skeletal malformations (mainly in the cardiovascular system, interventricular septal defects and levocardia) in rats treated with trichloroacetic acid (a metabolite of trichloroethylene) by gavage at maternal toxic dose levels. Cosby *et al*. (Cos92) observed no maternal, reproductive or developmental effects in mice treated by gavage with trichloroethylene at dose levels of up to 1/10 of the oral LD50. Taylor *et al*. (Tay85) described that trichloroethylene at a concentration of 1250 mg/l drinking water (near maximal solubility) induced effects on locomotor activity and exploratory behaviour in pups of rats. Cardiac defects and an increased number of implantation and resorption sites were observed in rats given drinking water containing trichloroethylene or trichloroacetic acid (Daw93, Joh98).

In conclusion, based on the observations of Dawson *et al*. (Daw93) and Johnson *et al*. (Joh98) about the increased incidence of cardiac defects after trichloroethylene or trichloroacetic acid treatment, the committee proposes to classify trichloroethylene in category 2 (substances that should be regarded as if they cause developmental toxicity in humans) and to label this compound with R61 (may cause harm to the unborn child). This conclusion is supported by the studies of Healy *et al*. (increased incidence of dams with total resorptions and pups showing delayed development), Dorfmueller *et al*. (minor malformations) and Smith *et al*. (embryolethality of TCA at maternally toxic doses) (Hea82) (Dor79) (Smi89). In the well-performed studies of Carney *et al*. (Car01)

and Fisher *et al*. (Fis01) no effects were observed on the incidence of heart malformations after treatment with trichloroethylene or TCA. However, these negative results were no reason for the committee to adjust the recommendation classifying in category 2. These differences in study outcomes might have been a result of differences in exposure routes or experimental design.

From the study of Fisher *et al*. (a pharmacokinetic lactation model), a concentration of 0.54 mg trichloroethylene/l breast milk was predicted (Fis97). The committee is of the opinion that this (predicted) trichloroethylene concentration in human breast milk can only be used as an indication for the possible amount of the compound in breast milk, because the model is not yet sufficiently validated. The committee concludes that the predicted exposure level does not warrant labelling. In rats, the concentration of trichloroethylene in milk after treatment by inhalation was 110 mg/l (highest estimated concentration was about 9 mg/l) (Fis90). In a drinking water study with rats, the concentration of trichloroethylene in milk was below the detection limit and the maximum concentration of trichloroacetic acid in milk van 1.5 mg/l (Fis90).

In conclusion, the committee is of the opinion that a lack of appropriate data precludes assessment of trichloroethylene for effects during lactation.

Proposed classification for fertility

A lack of appropriate human data precludes the assessment of trichloroethylene and sufficient animal data show that no classification for trichloroethylene is indicated

Proposed classification for developmental toxicity

Category 2, T;R61

Proposed labelling for effect during lactation

A lack of appropriate data precludes the assessment of trichloroethylene for effects during lactation

Additional consideration

The committee would like to emphasise that several human studies considered here in view of trichloroethylene exposure give reason for concern with respect to effects on fertility and development. However, it is not clear in these studies whether exposure involved pure trichloroethylene or a mixture of solvents containing trichloroethylene.

Therefore, the EU Classification and Labelling guideline does not warrant a classification of trichloroethylene on the basis of these human studies. However, the committee emphasises that there is clearly cause for concern for effects on fertility and development after exposure to mixtures of solvents containing trichloroethylene.

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A The Committee

B Comments on the public draft

C Directive (93/21/EEC) of the European Community

D Fertility and developmental toxicity studies

E Abbreviations

Annexes

Annex **A**

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Annex **B**

Comments on the public draft

A draft of the present report was released in 2002 for public review. The following persons and organisations have commented on the draft review:

- V Digernes, Federation of Norwegian Process Industries, Norway
- JW Wilmer, ESCA Occupational & Environmental Health Working group, Belgium

Annex **C**

Directive (93/21/EEC) of the European Community

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded as if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:

T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:

T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:

Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1 *Effects on male or female fertility*, includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would interfere

with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2 *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri/postnatal defects, and impaired postnatal, mental or physical development up to and including normal pubertal development.

Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity.

The placing of a compound in Category 1 for effects on Fertility and/or Developmental Toxicity is done on the basis of epidemiological data. Placing into Categories 2 or 3 is done primarily on the basis of animal data. Data from *in vitro* studies, or studies on avian eggs, are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

In common with most other types of toxic effect, substances demonstrating reproductive toxicity will be expected to have a threshold below which adverse effects would not be demonstrated. Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administrated, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in Category 3, or even no classification, will be warranted.

Annex V of the Directive specifies a limit test in the case of substances of low toxicity. If a dose level of at least 1000 mg/kg orally produces no evidence of effects toxic to reproduction, studies at other dose levels may not be considered necessary. If data are available from studies carried out with doses higher than the above limit dose, this data must be evaluated together with other relevant data. Under normal circumstances it is considered that effects seen only at doses in excess of the limit dose would not necessarily lead to classification as Toxic to Reproduction.

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would

lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification into Category 2 should only be made where there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it was demonstrated that impaired fertility in animal studies was due to failure to mate, then for classification into Category 2, it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposue is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as ‘Toxic to Reproduction’, unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

a toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or

b on the basis of results of one or two generation studies in animals which indicate the presence of adverse effects on the offspring due to transfer in the milk, and/or

c on the basis of evidence in humans indicating a risk to babies during the lactational period.

Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

Table 1.1 Fertility studies (inhalation) in animals with trichloroethylene.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/effects on reproduction	Remarks
Beliles *et al*. (1980)	Sprague Dawley rats (n=?)	7 h/d for 5 consecutive days	0,100 and 500 ppm (537 and 2685 mg^3) by inh	not presented	no effect on sperm head morphology	
Beliles *et al*. (1980)	CD-1 mice (n=?)	7 h/d for 5 consecutive days	0,100 and 500 ppm (537 and 2685 mg^3) by inh	not presented	increased anomalies in sperm head morphology after 1 and 4 w	
Slacik-Erben *et al* (1980)	male NMRI mice (n=50)	treatment for 24 h. Mating with untreated females for 4d. Females changed 12 times. Females sacrificed 13d after removal from male.	0,269, 1085, 2417 mg/m^3 by inh	not observed	no effects on fertility, pre-and post implantation loss, dominant lethal mutations	dominant lethal test
Land *et al*. (1981)	(C57B1/C3H) F1 male mice (n=5)	treatment for 5d, 4 h/d Sacrifice 28d after first exposure	0, 1074, 10740 mg/m^3 by inh	not presented	highest dose group: slight increase abnormal sperm	incidence of abnormal sperm in highest dose group within normal range
Maltoni *et al*. (1988)	Sprague Dawley rats (n=130-145/ sex)	7 h/d for 5 d/w for 104 weeks. Animals were kept under observations until spontaneous death	0, 100, 300 and 600 ppm (537, 1611 and 3222 mg^3) by inh	no effect of bw and mortality	a dose response related increase in Leydig cell tumours	no tumours after a 8 w treatment period no effect on Leydig cell tumours in mice

n=number of animals; h=hour; d=day; w=week; bw=body weight; inh=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day

Table 1.2 Fertility studies (gavage) in animals with trichloroethylene.

Authors	Species	Experimental period / design	Dose and route	General toxicity	Effects on reproductive organs/effects on reproduction	Remarks
Manson *et al*. (1984)	female Long Evans rats (n=23)	treatment during 2w premating, 1w mating [5d/w] and during 3w gestation [7d/w]. Treated females mated with untreated males. Sacrifice pn 31	0, 10, 100, 1000 mg/kg bw/d by gav	1000 mg: 3 rats died and BW gain reduced	1000 mg/kg bw/d: increased number of dead pups no effects on oestrus cycle nor on fertility	
Zenick *et al*. (1984)	male Long Evans rats (n=10)	treatment for 6w, 5 d/w. At the end of w1 and w5 and 4w post-exposure males mated with ovariectomixed females.	0, 10, 100 and 1000 mg/kg bw by gav	1000 mg: w1 impaired copulatory behaviour, no effects semen parameters; no effects in w5 and 4w after dosing	1000 mg/kg bw/d: w1 impaired copulatory behaviour, no effects semen parameters; no effects in w5 and 4w after dosing	effect on copulatory behaviour probably due to narcotic properties

n=number of animals; h=hour; d=day; w=week; bw=body weight; inh=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day

Table 1.3 Fertility studies (diet) in animals with trichloroethylene.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproductive organs/effects on reproduction	Remarks
Lamb *et al*. (1997a)	Swiss CD-1 mice (n=?)	study performed according to the Reproductive Assessment by Continuous Breeding protocol	0, 1.5, 3.0 and 6.0 g/kg diet (trichloroethylene microencapsulated in a gelatin and sorbitol shell	6.0 g/kg: hepatic and renal toxicity in males and females of both generations. Increased mortality in F1 generation	no treatment related effects on mating, fertility and reproduction 6.0 g/kg: sperm motility decreased in both generations	
Lamb *et al*. (1997b)	Fisher F344 rat (n=?)	study performed according to the Reproductive Assessment by Continuous Breeding protocol	0, 1.5, 3.0 and 6.0 g/kg diet (trichloroethylene microencapsulated in a gelatin and sorbitol shell	decreased bw: F0 females of all dose groups; F0 males of 6.0 g.kg group; F1 males and females of all dose groups increased kidney and liver weights in F0 males and females of 6.0 g/kg group increased liver weight in all F1 groups	decreased testis weight in all F1 groups	

n=number of animal; h=hour; d=day; w=week; bw=body weight; inh=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day

Table 2.1 Developmental toxicity studies (inhalation) with trichloroethylene.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/effects on reproduction	Rem.
Schwetz *et al*. (1975)	Swiss Webster mice (n=26 control and n=12 treatment group)	treatment gd 6-15 for 7 h/d sacrifice gd 18	0 and 1611 mg/m^3 by inh	no effects observed	no embryonal or foetal toxicity no teratogenic effects	
Schwetz *et al*. (1975)	Sprague Dawley rats (n=30 control and n=18 treatment group)	treatment gd 6-15 for 7 h/d sacrifice gd 18	0 and 1611 mg/m^3 by inh	1611 mg/m^3: decreased	no embryonal or foetal toxicity no teratogenic effects	
Dorfmueller *et al*. (1979)	Long Evans rats (n=8-12)	2 w premating (5 d/w, 6h/d) gestation (7 d/w, 6h/d) alternate air or trichloroethylene sacrifice d 20	0 and 9666 mg/m^3 by inh	no effects observed	9666 mg/m^3 during pregnancy: delayed skeletal maturation 9666 mg/m^3 during premating: reduction in postnatal bw no signs of embryo toxicity, teratogenicity or behavioural deficits	
Beliles *et al*. (1980)	Sprague Dawley rats (n=30)	3 w premating (half of the groups) and gd 0-18 or 6-18 (5d/w, 7h/d). sacrifice gd 21	0 and 2685 mg/m^3 by inh	no effects observed	no foetal toxic or teratogenic effects	
Beliles *et al*. (1980)	New Zealand White rabbits (n=24-28)	3 w premating (half of the group) and gd 0-21 or 7-21 (5d/w, 7h/d). sacrifice gd 30	0 and 2685 mg/m^3 by inh	no effects observed	no foetal toxic effects toxic effects two fetuses in 2 litters showed (external) hydrocephaly	
Healy *et al*. (1982)	Wistar rats (n=31 control and n=32 treatment group)	treatment during gd 8-21 (4 h/d) sacrifice gd 21	0 and 537 mg/m^3 by inh	no effects observed	537 mg/m^3: 7 dams with total resorptions (control group 2). reduced foetal skeletal maturation no external, visceral or skeletal malformations.	
Carney *et al* (2001)	CD rats (n=27 per group)	treatment during gd 6-20, 6h/day. sacrifice on gd 21	0, 50, 150 and 600 ppm by inh. (0, 269, 806 and 3222 mg/m^3	600 ppm decreased body weight gain dams on day 6-9. 50 and 150 ppm: no effects	no effects at any dose level	

n=number of animal; h=hour; d=day; w=week; bw=body weight; inh=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day

Table 2.2 Developmental toxicity studies (gavage) with trichloroethylene.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity	Remarks
Kavlock *et al*. (1979)	CD-1 mice (n=19-84)	treatment from gd 7-14 sacrifice gd 18	rats were treated by gav with organic materials concentrated from drinking water at dose levels 300, 1000 and 3000 times the anticipated human exposure.	slight effects on BW and rel. liver weight	no skeletal or visceral effects observe	calculated dose of trichloroethylene: 0.02 mg/kg bw/day simultaneous treatment to other compounds (e.g. chloroform)
Smith *et al*. (1989)	Long Evans rats (n=20-26)	treatment from gd 6-15 sacrifice gd 20	0, 330, 800, 1200, 1800 mg/kg bw/d trichloroacetic acid by gav	$\geq$ 800 mg/kg: reduced maternal weight gain $\geq$ 330 mg/kg: increased spleen and kidney weight	$\geq$ 800 mg/kg: embryo lethality $\geq$ 330 mg/kg: foetal weight and foetal length decreased $\geq$ 330 mg/kg: soft tissue malformations in cardiovascular system $\geq$ 1200: skeletal malformations	trichloroacid acid is one of the major metabolites of trichloroethylene
Cosbey *et al*. (1992)	B6D2F1 mice (n=12-13)	treatment from gd 1-5, 6-10, 11-15 sacrifice: pn d 15 or 22	0, 24, 240 mg/kg bw/d by gav (=1/100 or 1/10 LD 50)	no maternal toxic effects observed	no reproductive or developmental effects	
Fisher *et al* (2001)	CDR(CD) Sprague Dawley rats	treatment from gd 6-15 sacrifice day 21 (n=19 or 20 per group)	TCE: 500 mg/kg bw/day TCA: 300 mg/kg bw/day DCA: 300 mg/kg bw/day	maternal body weight gain was sign. less in the TCA and DCA group. no effect in TCE group	no increased heart malformations per litter or fetus	

n=number of animal; h=hour; d=day; w=week; bw=body weight; inh=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day, TCE=trichloroethylene, TCA=trichloroacetic acid, DCA=dichloroacetic acid

Table 2.3 Developmental toxicity studies (drinking water) with trichloroethylene.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity	Rem.
Taylor *et al*. (1985)	Sprague Dawley rats (n=?)	2w prem, mating, gestation and lactation d21, sacrifice pups d 90	0, 312, 625 and 1250 mg/l in drinking water	not presented	312 and 625 mg/l: exploratory behaviour 60 and 90 day male pups increased 1250 mg/l: exploratory behaviour 60 and 90 day male pups increased and locomotor activity 60-day old male pups increased	

Dawson *et al*. (1993)	Sprague Dawley rats (n=?)	treatment: 2m before gestation or 2 m before and during gestation or during gestation	0, 1.5 and 1100 mg/l in drinking water	no effects observed	before gestation: no effects before and during gestation: increased number of foetal heart defects at both dose levels during gestation: increased number of foetal heart defects at highest dose level
Johnson *et al*. (1998)	Sprague Dawley rats (n=8-55)	treatment during gestation sacrifice on gd 22	metabolites of trichloroethylene in drinking water at various concentrations	no effects observed	no effect on reproductive parameters and noncardiac developmental parameters except for an increased number of implantation- and resorption sites (per litter) in the trichloroacetic acid group. In this group foetuses showing cardiac defects were observed. control (implantations per litter):0.2 TCA (implantations per litter): 1.1 control (resorptions per litter): 0.7 TCA (resorptions per litter): 2.7

n=number of animal; h=hour; d=day; w=week; bw=body weight; inh=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day, TCA=trichloroacetic acid

Table 2.4 Developmental toxicity studies (diet) with trichloroethylene.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Developmental toxicity	Rem.
Lamb *et al*. (1997a)	Swiss CD-1 mice (n=?)	study performed according to the Reproductive Assessment by Continuous Breeding protocol	0, 1.5, 3.0 and 6.0 g/kg diet (trichloroethylene microencapsulated in a gelatin and sorbitol shell)	6.0 g/kg: hepatic and renal toxicity in males and females of both generation increased mortality in F1 generation	pup body weight reduced. Increased perinatal pup mortality	
Lamb *et al*. (1997b)	Fisher F 344 rat (n=?)	study performed according to the Reproductive Assessment by Continuous Breeding protocol	0, 1.5, 3.0 and 6.0 g/kg diet (trichloroethylene microencapsulated in a gelatin and sorbitol shell)	decreased BW: F0 females of all dose groups; F0 males of 6.0 g.kg group; F1 males and females of all dose groups Increased kidney and liver weights in F0 males and females of 6.0 g/kg group increased liver weight in all F1 groups	F0-generation: decreased litter sizein 3.0 and 6.0 g/kg groups F1-generation: decreased pup weights in all dose groups	

n=numer of animal; h=hour; d=day; w=wek; bw=body weight; ing=inhalation; ip=intraperitoneal; gav=gavage; pn=post-natal; gd=gestation day

Annex **E**

Abbreviations

Abbreviations used:

bw	=	body weight
d	=	day
F	=	female(s)
i.p.	=	intraperitoneal
i.v.	=	intravenous
M	=	male(s)
n	=	number
NOAEL	=	no adverse effect level
OECD	=	Organisation for Economic Cooperation and Development
PN	=	postnatal